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# CADMIUM EFFECTS ON INFLUX AND TRANSPORT OF MINERAL NUTRIENTS IN PLANT SPECIES

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***ABSTRACT:*** Solution culture experiments were conducted under controlled environmental conditions to determine the effects of cadmium(II) [Cd(II)] activity (0, 8, 14, 28, 42, and 54 µM) on influx (IN) into roots and transport (TR) from roots to shoots of zinc (Zn), copper (Cu), iron (Fe), manganese (Mn), calcium (Ca), magnesium (Mg), phosphorus (P), and sulfur (S) in ryegrass (*Lolium perenne* L.), maize (*Zea mays* L.), white clover (*Trifolium repens* L.), and cabbage (*Brassica oleracea* var. *capitata* L.). Shoot and root dry matter (DM) decreased with increased external Cd, and plant species differed extensively. Ryegrass and cabbage were relatively tolerant to Cd toxicity compared to white clover and maize. Influx and TR of Cu, Zn, Fe, Mn, Ca, and Mg were lower with increasing external Cd compared to controls, and species also differed. Influx and TR of P were enhanced in each species with up to 14 µM Cd, decreased in white clover and cabbage at higher Cd levels, while in maize and ryegrass continued to increase as Cd increased. Influx and TR of S were high in white clover at 8 µM Cd and decreased as Cd increased. Influx of S was high in ryegrass, but TR of S remained relatively constant as Cd increased. Influx and TR of S did not significantly change in maize, but decreased in cabbage as Cd increased. With Cd up to 14 µM,

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decreases in both IN and TR of Zn, Fe, Mn, Ca, and Mg were greater in white clover than in cabbage. Sensitivity of the dicotyledonous plant species to Cd toxicity might have been associated with Cd effects on IN and TR of Fe, Mn, Ca, and Mg. However, differences in plant sensitivities to Cd toxicity between ryegrass and maize were not reflected in Cd effects on IN and TR of mineral nutrients.

## INTRODUCTION

Cadmium is not an essential element for plant growth, but is toxic to plants, animals, and humans. In general, Cd toxicity symptoms in plants are leaf chlorosis and necrosis followed by leaf abscission, and purpling beginning at the stem of soybean [*Glycine max* (L.) Merr] leaves (Foy et al., 1978). Toxicity symptoms result from disturbances of many important physiological processes and interferences in uptake and transport of mineral nutrients. For example, sunflower (*Helianthus annuus* L.) and maize plants grown with Cd had inhibited net photosynthesis (Bazzaz et al., 1974; Miller et al., 1973). Inhibited photosynthetic carbon dioxide ($CO_2$) fixation and non-cyclic electron transport were also noted in spinach (*Spinacia oleracea* L.) plants (Hampp et al., 1976), likely because of impairment of photosynthesis II coupling (Li and Miles, 1975). In addition, chlorophyll concentrations in leaves decreased in leaves of cucumber (*Cucumus sativus* L.) grown with Cd (Klobus and Buczek, 1985).

Cadmium toxicity has been related to interactions in uptake and translocation of mineral nutrients in plants. Leaf chlorosis from excess Cd appeared to be associated directly or indirectly with Fe (Foy et al., 1978; Root et al., 1975) or Zn deficiencies (Turner, 1973). Both synergistic (Turner, 1973; Smith and Brennan, 1983) and antagonistic (Root et al., 1975; Abdel-Sabou et al., 1988) Cd-Zn interactions have been reported in plants. High Cd levels in soils decreased Zn uptake in *Brassica chinensis* (Wong et al., 1984), but addition of Cd had no antagonistic effects on Zn uptake in flax (*Linum usitatissimum* L.) (Moraghan, 1993). Kitagishi and Yamane (1981) reported synergistic Cd-Zn interactions in rice (*Oryza sativa* L.), and Lagerwerff and Biersdortf (1972) noted antagonistic interactions between these elements in uptake/transport studies.

Different effects of Cd on uptake of Fe, Cu, and Mn have been reported. Addition of Cd to growth media both enhanced (Wallace et al., 1980; Sela et al., 1988) and depressed (Khan and Khan, 1983; Bjerre and Schierup, 1985) Fe accumulation in plants. High Cd levels increased Fe concentrations in *Brassica chinensis* shoots (Wong et al., 1984). Cadmium was also reported to cause Fe deficiency in plants (Root et al., 1975). Effects of Cd on uptake of Cu and Mn were either synergistic (Khan and Khan, 1983; Vasquez et al., 1989) or antagonistic (Keck, 1978; Bjerre and Schierup, 1985). Root absorption and transfer of Cd from roots to shoots were inhibited by Fe, Cu, and Mn in soybean (Cataldo et al., 1984). Cadmium also depressed Mn uptake in several plants (Wallace et al., 1977; Root et al., 1975).



Information about Cd effects on IN and TR of Ca, Cu, Fe, Mg, Mn, P, S, and Zn in different plant species is limited. Likewise, information is lacking on effects of toxic levels of Cd on uptake and translocation of essential mineral nutrients relative to plant tolerance to Cd. Differences noted for interactions of Cd with mineral nutrients may have been because of plant species and cultivar variabilities, nature of growth media, and Cd levels used. Previous studies indicated that sensitivity of plant species to Cd toxicity was correlated with IN and TR of Cd (Yang et al., 1995).

The objectives of this study were to determine effects of Cd(II) activity on IN and TR of Ca, Cu, Fe, Mg, Mn, P, S, and Zn in four agriculturally important crop species, and to better understand interactions of Cd with mineral nutrients relative to Cd tolerance of plant species.

## MATERIALS AND METHODS

### Plant Culture

White clover (*Trifolium repens* L., cv 'California Ladino F-8339 G') seeds were scarified with sand paper, sterilized with 5% NaOCl (household bleach) for 5 min and washed numerous times with distilled water. Seeds of the other plant species [cabbage (*Brassica oleracea* var. capitata L., cv 'Early Jersey Wakefield'), maize (*Zea mays* L., cv 'Early Sunglow'), and ryegrass (*Lolium perenne* L., cv 'Linn')] were likewise sterilized. Sterilized seeds were wrapped in germination papers and germinated with aerated 0.1-strength nutrient solution. Seedlings were transferred to containers with full-strength nutrient solution and grown in a growth chamber at 25/20°C, 60/70% relative humidity, and 14/10 h (light/dark) with a photosynthetic photon flux of 400 µmol/m/s using incandescent and fluorescent (Sylvania Cool White VHO 215 W) lamps. Composition of full-strength nutrient solution was 4.2 $NO_3$-N, 0.4 $NH_4$-N, 2.0 Ca, 0.5 Mg, 1.0 K, 0.1 P, and 1.7 S in mM; and 4.7 Mn, 6.6 B, 0.6 Zn, 0.2 Cu, 0.1 Mo, 9.4 Cl, 0.2 Na, and 20 Fe as Fe-EDDHA [ferric N,N'-ethylene bis(2-(2-hydroxyphenyl) glycine)] in µM.

White clover was 32-days-old, ryegrass 30-days-old, cabbage 21-days-old, and maize 14-days-old when plants were introduced to Cd treatments. Numbers of plants/pot in Cd treatments were 108 for ryegrass, 72 for white clover, 36 for cabbage, and 18 for maize. Plants were introduced to Cd treatments of 0, 8, 14, 28, 42, and 54 µM as $CdCl_2$ in 6-L plastic pots containing full-strength nutrient solution at pH 5.5. Activities of Cd(II) in nutrient solutions were calculated by a GEOCHEM program (Parker et al., 1995). Nutrient solutions with Cd had pH adjusted daily (5.5) and were replaced once during the treatment period (after seven days). Pots were completely randomized with three replications, and each plant species was grown at a different time. Plants were grown in Cd treatments for 14 d before experiments were terminated.



**Plant Analysis**

Plants were harvested at the start and end of Cd treatments. At harvest, roots of intact plants were immersed in 20 mM $Na_2$-EDTA (disodium ethylenediamine-tetraacetate) for 15 min to remove Cd adhering to root surtaces. Shoots were separated from roots, base of stalks rinsed with bidistilled water, dried at 65°C and weighed. Roots were rinsed thoroughly with bidistilled water, blotted dry, cut into 1-2 cm segments, 2 g representative fresh samples collected for root length measurements using a Comair root length scanner (Commonwealth Aircraft Corp., Melbourne, Australia[4]), and remainder of roots were dried and weighed. Dried shoot and root samples were ground to pass 0.55-mm sieve using a UDY Cyclone Sample Mill (UDY Corp., Fort Collins, CO[4]). Subsamples (100 mg) were weighed into plastic containers, nitric acid (1.0 mL 15.6M $HNO_3$) was added, containers placed in Parr microwave acid digestion bombs (Parr Instrument Co., Moline, IL[4]), microwaved for 4 minutes at 70% power, cooled, transferred to new containers, diluted to 10.0 mL with bidistilled water, and filtered. Concentrations of Cd were determined by graphite furnace atomic absorption spectroscopy (Perkin-Elmer, Norwalk, CT[4]), and the other mineral elements by inductively coupled plasma spectroscopy (Applied Research Labs., Dearborn, MI[4]).

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**Calculation of Influx and Transnort of Mineral Nutrients**

Influx (IN) into roots and transport (TR) from roots to shoots were calculated (Baligar et al., 1993):

$$IN = [(PU_2 - PU_1)/(t_2 - t_1)] \times [(\ln RL_2 - \ln RL_1)/(RL_2 - RL_1)] \quad [1]$$

$$TR = [(SU_2 - SU_1)/(t_2 - t_1)] \times [(\ln SW_2 - \ln SW_1)/(SW_2 - SW_1)] \quad [2]$$

where: **PU** = Cd uptake by whole plant (mmol Cd/plant); **RL** = root length (m/plant); **SU** = Cd uptake by shoots (mmol Cd/plant); **SW**=shoot dry weight (g/plant); Subscripts **1 & 2** =sampling time at the start and at the end of Cd treatment, respectively; and **t** = time (d).

IN was converted to pmol/cm RL/s and TR to nmol/g shoot dry weight/s.



## RESULTS AND DISCUSSION

### Plant Growth

Ryegrass and cabbage shoot DM decreased about 10-20% that of controls, and maize and white clover shoot DM decreased ~50% that of controls when plants were grown with Cd up to 14 µM (Fig. 1). White clover root DM decreased ~80% that of controls, and cabbage ~50% that of controls when plants were grown with Cd up to 14 µM. Cadmium was more deleterious to root than to shoot growth for each plant species. On the basis of DM, ryegrass and cabbage were relatively tolerant to Cd toxicity. while white clover and maize were relatively sensitive to Cd toxicity when plants were grown with Cd up to 14 µM.

### Cadmium Toxicity Symptoms

Visible Cd toxicity symptoms were observed on leaves of white clover and maize grown 14 d with 8 µM Cd, for cabbage grown with 14 µM, and for ryegrass grown with 28 µM (Table 1). Typical Cd toxicity symptoms for white clover were reddish-brown spots on old and young leaves between veins with symptoms moving from leaf margin toward the central leaf vein over time. New leaves ceased to grow when severe toxicity symptoms appeared. Older leaves of ryegrass turned brownish-yellow with some reddish-brown spots appearing from leaf tip to base. Young leaves remained green, even though growth was inhibited. Older maize leaves become chlorotic, and chlorosis developed from leaf tip to base between veins. New leaves curled with development of severe chlorosis along the middle

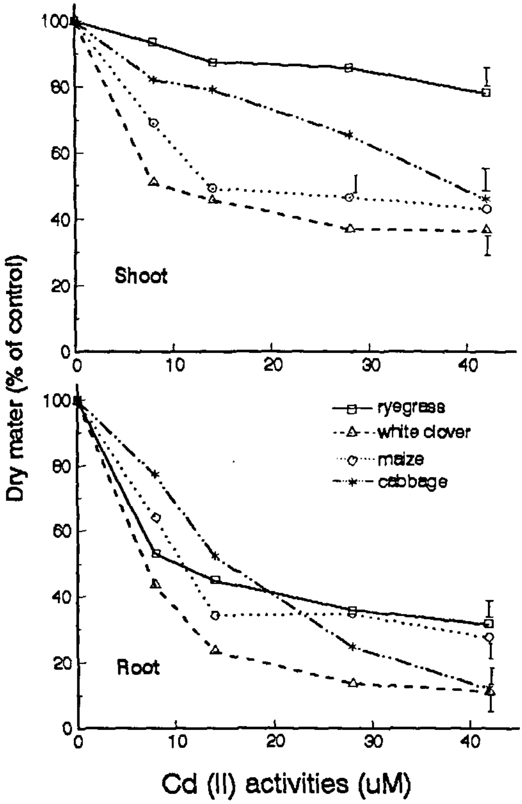


**FIGURE 1.** Shoot and root dry matter (DM) of four plant species grown with different Cd(II) activities in nutrient solution. Vertical bars are LSD ($P<0.05$) for the corresponding line of data. Shoot DM of controls were 0.21, 0.23, 0.87, 0.55, and root DM of controls were 0.13, 0.08, 0.31, and 0.12 g/plant for ryegrass, white clover, maize, and cabbage, respectively.

**TABLE 1.** Severity of Cd toxicity symptoms on four plant species grown 14 d with Cd treatments†.

| **Cd Level** | **Severity of Toxicity Symptoms on Shoots** | | | |
|---|---|---|---|---|
| **(µM)** | **White Clover** | **Ryegrass** | **Maize** | **Cabbage** |
| Control | -- | -- | -- | -- |
| 8 | ++ | -- | + | -- |
| 14 | +++ | -- | + + | + |
| 28 | ++++ | + | ++ | ++ |
| 42 | +++++ | + | +++ | +++ |

† = --, +, + +, +++, ++++, and + + + + + refer to symptoms where 0, 0-10%, 10-30%, 30-50%, 50-70%, and 70-90% of leaves exhibited toxicity symptoms, respectively.



vein, and eventually died. Cabbage leaves curled and wilted with some chlorotic spots developing between leaf veins, and purple appeared on the underside of leaves; first on older leaves and progressing to younger leaves. Growth of young leaves was inhibited.

**Influx of Mineral Nutrients**

Influx of Zn, Fe, Cu, Mn, Ca, and Mg decreased with increasing external Cd levels, and plant species differed (Figures 2 and 3). Influxes decreased 35% for Zn, 40% for Fe, 80% for Mn, 40% for Ca, and 75% for Mg in white clover grown with Cd up to 14 µM compared to controls. Influx of Zn, Fe, and Mn was reduced only ~20% compared to controls, and no reduction in IN of Ca and Mg was noted in cabbage. The decrease in IN of Cu was relatively small for both white clover and cabbage grown with Cd up to 14 µM compared to controls (Fig. 2). Accordingly, white clover was relatively sensitive to Cd toxicity compared to cabbage when grown with Cd up to 14 µM. Toxicity of Cd in these two dicotyledonous species may have been associated with inhibition of Zn. Fe, Mn, Ca, and Mg uptake by Cd, especially Mn and Mg at 14 µM Cd.

Influxes decreased 10% for Zn, 30% for Fe, 20% for Cu, 70% for Mn, 60% for Ca, and 50% for Mg compared to controls in ryegrass, and 25% for Zn, 10% for Fe, 1% for Cu, 90% for Mn, 55% for Ca, and 35% for Mg compared to controls in maize when plants were grown with Cd up to 14 µM (Figures 2 and 3). No consistent associations between sensitivity of plants to Cd toxicity and influence

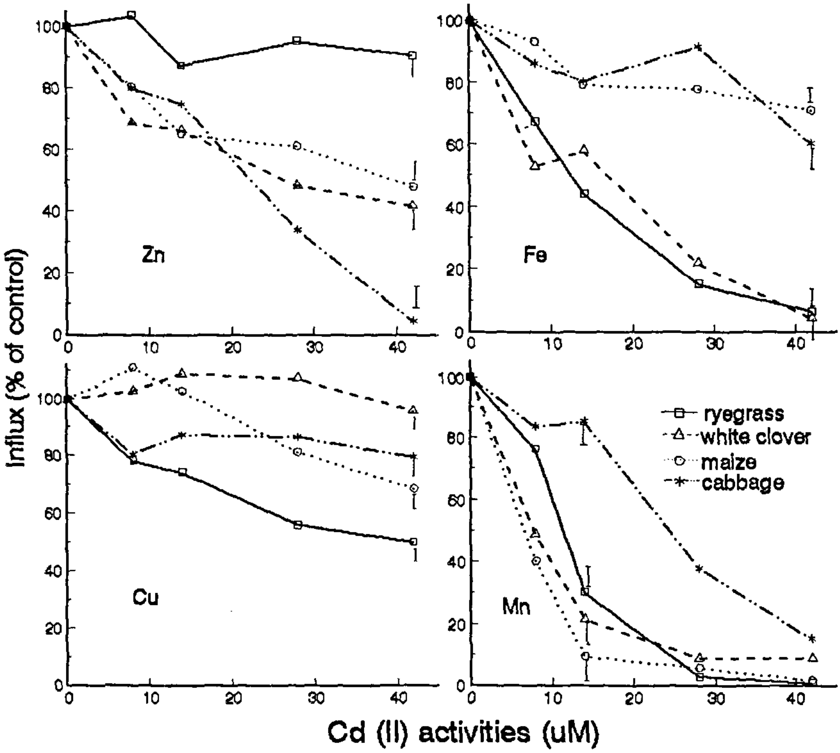


**FIGURE 2.** Influx (IN) of Zn, Cu, Fe, and Mn into roots of four plant species grown with different Cd(II) activities in nutrient solution. Vertical bars are LSD ($P<0.05$) for the corresponding line of data. Influx (pmol/cm/s) of controls were: Zn = 0.0141, 0.0229, 0.0370, and 0.0154; Cu = 0.0195, 0.0069, 0.0138, and 0.0048; Fe = 0.0835, 0.0754, 0.112, and 0.0874; and Mn = 0.0444, 0.0665, 0.0901, and 0.0617 for ryegrass, white clover, maize, and cabbage, respectively.

of Cd on IN of Cu, Fe, Mn, Ca, and Mg were noted for maize and ryegrass, even though ryegrass was relatively more tolerant to Cd toxicity than maize.

Influx of P continued to increase in maize and ryegrass grown with increasing external Cd from 0 to 42 μM compared to controls, but white clover peaked at 14 μM and cabbage peaked at 28 μM (Fig. 3). Influx of S increased in ryegrass grown with external Cd up to 42 μM compared to controls and white clover grown with Cd at <28 μM (Fig. 3). Influx of S in maize remained unchanged while IN of S in

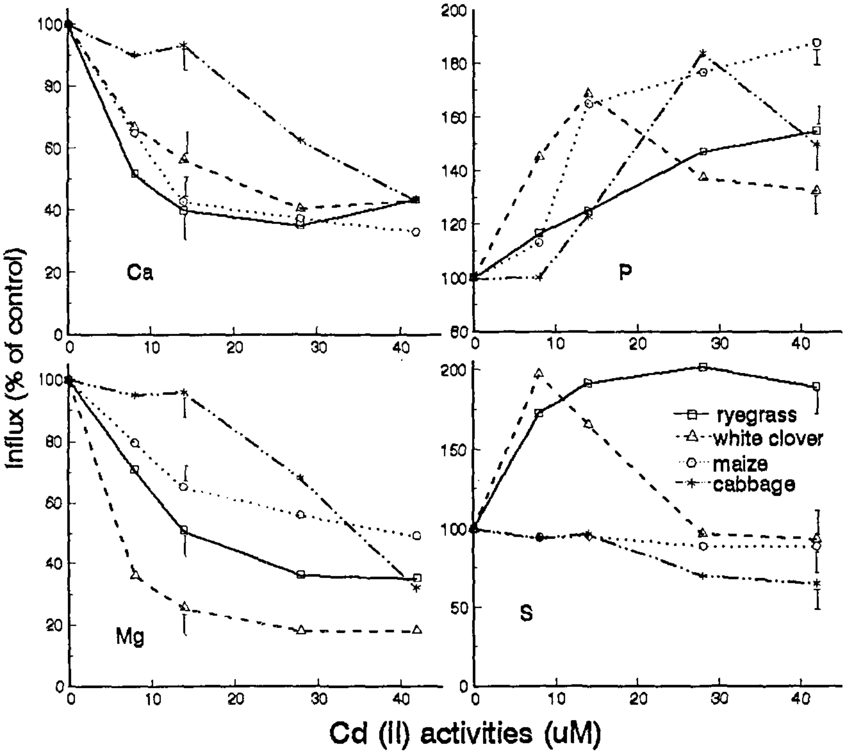


**FIGURE 3.** Influx (IN) of Ca, Mg, P, and S into roots of four plant species grown with different Cd(II) activities in nutrient solution. Vertical bars are LSD ($P<0.05$) for the corresponding line of data. Influx (pmol/cm/s) of controls were: Ca = 6.335, 12.84, 8.321, and 23.011; Mg = 3.849, 5.69, 5.749, and 5.025; P = 0.871, 1.913, 1.412, and 1.112; and S = 1.758, 3.375, 3.243, and 13.326 for ryegrass, white clover, maize, and cabbage, respectively.



cabbage declined slightly for plants grown with Cd>14 μM. Ryegrass tolerance to high Cd might have been related to high IN of S, since S-rich compounds like polypeptides (phytochelatin) have been associated with detoxification of Cd (Robinson, 1990).

## Transport of Mineral Nutrients

Transport of Zn, Fe, Cu, Mn, Ca and Mg decreased compared to controls with increasing Cd in solution, and species differed (Figures 4 and 5). Transport of Fe,

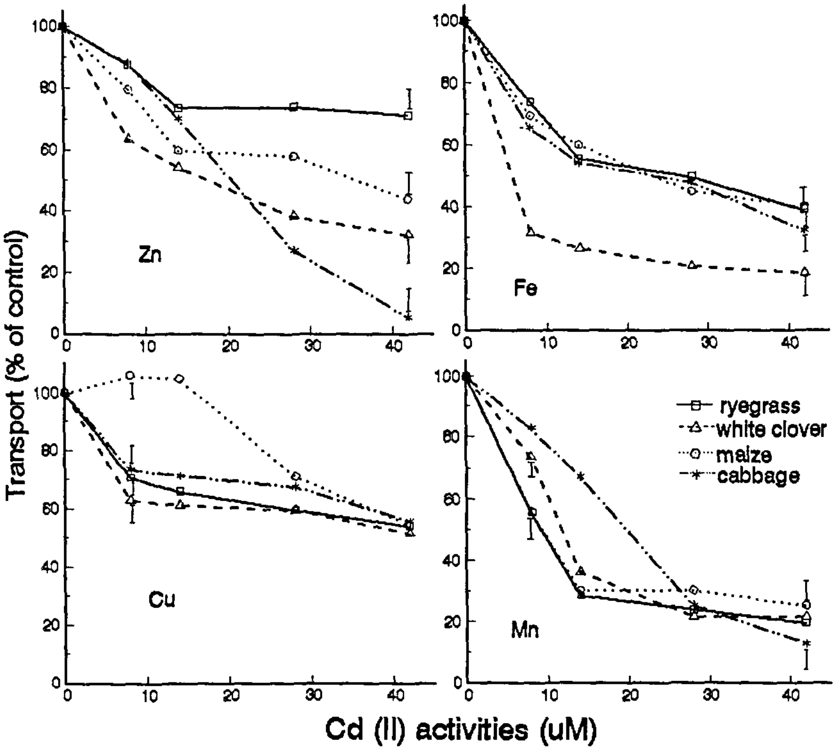


FIGURE 4. Transport (TR) of Zn, Cu, Fe, and Mn from roots to shoots of four plant species grown with different Cd(II) activities in nutrient solution. Vertical bars are LSD ($P<0.05$) for the corresponding line of data. Transport (nmol/g/s x $10^3$) of controls were: Zn = 0.89, 1.67, 2.22, and 0.88; Cu = 0.77, 0.56, 0.77, and 0.35; Fe = 3.62, 6.29, 3.44, and 0.35; and Mn 3.75, 3.90, 4.46, and 4.96 for ryegrass, white clover, maize, and cabbage, respectively.



Mn, Ca and Mg decreased 55-70% and Cu and Zn 40-60% in white clover plants grown with Cd up to µM compared to controls. Transport decreased by 30% for Zn, 55% for Fe, 30% for Mn, 30% for Cu, and 20% for Mg when cabbage was grown with Cd up to 14 µM. In addition, shoot Mn concentrations decreased from 94.5 to 52.8 mg/kg in white clover and from 157 to 126 mg/kg in cabbage. Shoot concentrations of Mg decreased from 2.67 to 1.52 mg/g in white clover and only from 4.61 to 4.43 mg/g in cabbage, and Ca decreased from 23.05 to 3.37 mg/g in

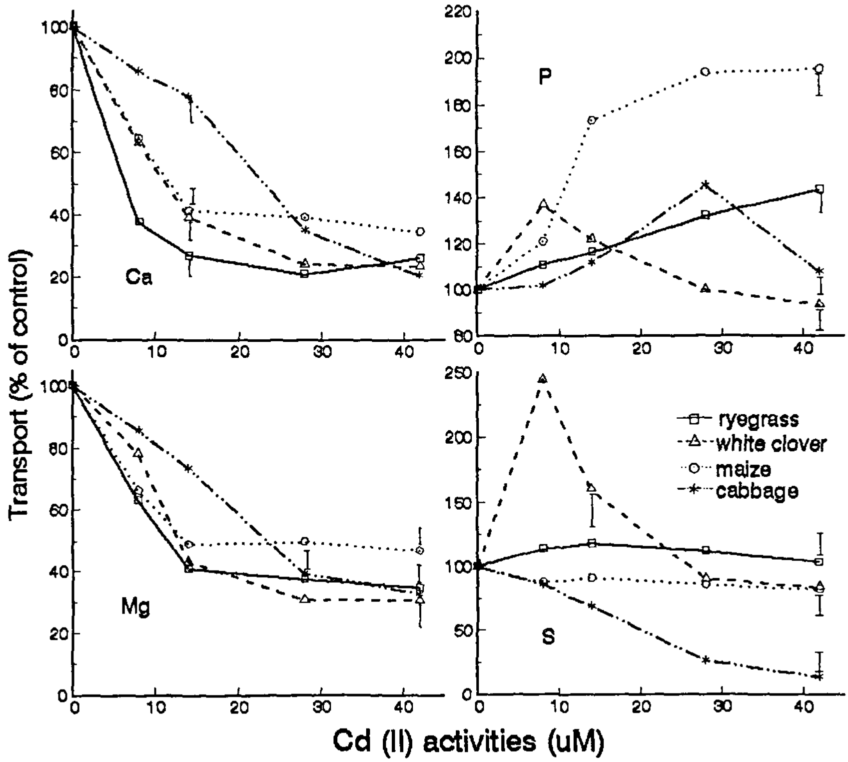


FIGURE 5. Transport (TR) of Ca, Mg, P, and S from roots to shoots of four plant species grown with different Cd(II) activities in nutrient solution. Vertical bars are LSD ($P<0.05$) for the corresponding line of data. Transport (nmol/g/s x $10^3$) of controls were: Ca = 0.55, 1.31, 0.39, and 1.85; Mg = 10.03, 9.31, 11.85, and 134.32; P = 4469.0, 0.17, 687.1, and 6349.2; and S = 0.124, 0.196, 0.155, and 0.826 for ryegrass, white clover, maize, and cabbage, respectively.

white clover and only from 42.6 to 38.4 mg/g in cabbage when plants were grown with 14 μM Cd. The sensitivity of white clover to Cd toxicity might have been associated with inhibition of TR of these mineral nutrients to induce Mn, Mg, and/or Ca deficiencies.

Transport of Zn, Fe, Mn, Ca, and Mg decreased similarly in maize and ryegrass grown with Cd up to 14 μM (Figures 4 and 5), even though ryegrass was relatively more tolerant to Cd than maize. Both Mn and Mg concentrations decreased markedly compared to controls (Mn decreased from 134.7 to 58.4 mg/kg in ryegrass and from 143.8 to 91.6 mg/kg in maize and Mg decreased from 3.40 to 1.67 mg/g in ryegrass and only from 3.84 to 3.43 mg/g in maize) when plants were grown with up to 14 μM Cd. In contrast, Cd toxicity symptoms appeared on ryegrass only when plants were grown 14 d with 28 μM Cd, while maize plants had toxicity symptoms when grown 14 d with 8 μM Cd. The differences in ryegrass and maize sensitivity to Cd toxicity did not appear to be associated with Cd effects on IN and TR of Zn, Fe, Mn, Cu, Ca, and Mg.

Transport of P increased 2-fold in maize, and only ~30% in the other species grown with Cd up to 28 μM (Fig. 5). Transport of S increased considerably in white clover grown with 8 μM Cd, but decreased with increased Cd to >28 μM. Transport of S in cabbage decreased consistently to near 25% that of controls as Cd increased to 42 μM. Excess solution Cd had little effect on TR of S in ryegrass and maize. Differences among plant species for sensitivity to Cd toxicity did not appear to be associated with TR of P and S.



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